[CONTRIBUTION FROM THE RADIATION LABORATORY AND DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY][1]

The Synthesis of Leucine and Several Branched Chain Fatty Acids Labeled with Carbon-14 in Various Positions

BY H. HAUPTMANN,[2] P. T. ADAMS AND B. M. TOLBERT

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The following compounds have been synthesized labeled with carbon-14. The yields, based on labeled carbon dioxide used, are indicated in parentheses: sodium isovalerate-1-C^{14} (96.0); sodium isocaproate-1-C^{14} (95.5); sodium isocaproate-2-C^{14} (39); DL-leucine-1-C^{14} (43.5); and DL-leucine-2-C^{14} (29.3). A novel method was developed for the preparation of alcohols from intermediate weight fatty acids, and by this method isoamyl bromide-1-C^{14} was prepared in 73% yield based on sodium isovalerate-1-C^{14} used.

The synthesis of a number of branched chain fatty acids and leucine labeled with carbon-14 in various positions was undertaken for a series of biological studies. By carbonation of the corresponding alkyl Grignard, two carboxyl-labeled acids were prepared: sodium isovalerate-1-C^{14} (96% yield) and sodium isocaproate-1-C^{14} (95% yield).[3]

Sodium isocaproate-2-C^{14} was prepared from isovaleric-1-C^{14} acid *via* the alcohol, bromide and nitrile by a method that involved a novel approach to the problem of preparing pure alcohols and halides in good yields on a small scale.

$$(CH_3)_2CH_2CH_2C^{14}O_2Na \xrightarrow[280°]{\text{cetyl bromide}} (CH_3)_2CH_2CH_2C^{14}O_2C_{16}H_{33} \xrightarrow[\text{copper chromite}]{H_2} (CH_3)_2CH_2CH_2C^{14}H_2OH \xrightarrow{PBr_3} (CH_3)_2CH_2CH_2C^{14}H_2Br \xrightarrow[(2)\ OH^-]{(1)\ KCN} (CH_3)_2CH_2CH_2C^{14}H_2CO_2Na$$

The cetyl ester was prepared in high yields (> 95%) by the direct reaction of sodium salt of the acid and cetyl bromide. This product could then be reduced over copper chromite to give a mixture of the two alcohols. The large differences in the boiling point of these two compounds made possible the easy isolation of the low-boiling component in excellent yields in a relatively pure state.

Even though preparation of the alcohol is possible in a one-step process in excellent yields by lithium aluminum hydride reduction of the acid,[4] the product is often difficult to separate from traces of inactive organic impurities derived from the solvents employed in the reaction.[5] The absence of any organic solvent in the esterification and reduction of the isovalerate eliminated the problem of contamination of the alcohol.

The conversion of the isoamyl bromide to the isocaproic acid was first attempted by formation of the Grignard of the halide, followed by carbonation at −25°. The yields, however, were very low (about 40%) so the method *via* the nitrile which gave yields of 55–65% was used. By bromination and amination of the corresponding labeled isocaproic acid, DL-leucine-1-C^{14} and DL-leucine-2-C^{14} were prepared.

The radio purity of the amino acids was established by two dimensional paper chromatography[6] using a butanol–propionic acid–water mixture in one direction and phenol–water in the other. Radioautographs of the paper chromatogram showed only one radioactive spot and when the paper itself was sprayed with ninhydrin, only one amino acid spot was observed. The identity of the acids was checked by equivalent weight determinations, and their radio purity established by one-dimensional paper chromatograms in water–ammonia–propanol solutions.

Experimental

Isoamyl-1-C^{14} Bromide.—Sodium isovalerate-1-C^{14} (2.10 g., 16.9 millimoles, 7.09 mc. total activity) was added to an ignition tube containing 5.40 g. of cetyl bromide (17.7 millimoles). The tube was sealed and heated with shaking at 280° for 11 hours. After reaction the ignition tube contents were dissolved in ether and filtered. An ether-insoluble, water-soluble residue containing 0.05 mc. of carbon-14 was left on the filter paper. The filtrate was washed into a 200-ml. stainless steel reaction vessel[7] and warmed to 40–50° to evaporate most of the ether. After addition of 5.5 g. of copper chromite[8] the reaction vessel was closed, evacuated, and maintained at a pressure of 50 microns for 1.5 hours to remove traces of ether. The reaction vessel was filled to a pressure of 170 atmospheres with hydrogen and heated at 250° with shaking for 10 hours. The hydrogen was released through a spiral trap equipped with a sintered disk[9] and cooled with liquid air. The reaction vessel was then evacuated and maintained at reduced pressure overnight while warmed to approximately 80° and the distillate was collected in a trap cooled in liquid nitrogen.

The alcohol thus obtained was converted to the bromide with phosphorus tribromide.[5b] The isoamyl bromide was purified by washing with water and drying over phosphorus pentoxide; yield 1.88 g., or 73% based on the isovalerate used.

Isocaproic-2-C^{14} Acid.—The isoamyl bromide (1.88 g., 12.4 millimoles) was distilled into a vessel containing 1.65 g. of potassium cyanide (25 millimoles) and 0.2 g. of potassium iodide in 25 ml. of ethanol. The reaction mixture was allowed to reflux 42 hours, at which time it was cooled and 4 g.

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(2) Rockefeller Fellow. While on leave from the Faculdade de Filosofia, Ciencias e Letras, Universidade de Sao Paulo, Sao Paulo, Brazil.

(3) (a) B. M. Tolbert, W. G. Dauben and J. C. Reid, *Anal. Chem.*, **21**, 1014 (1949); (b) M. Calvin, *et al.*, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, pp. 142, 179.

(4) R. F. Nystrom and W. G. Brown, THIS JOURNAL, **69**, 2548 (1947).

(5) (a) J. D. Cox, H. W. Turner and R. J. Warne, *J. Chem. Soc.*, 3167 (1950); (b) R. Ostwald, P. Adams and B. Tolbert, THIS JOURNAL, **74**, 2425 (1952).

(6) A. A. Benson, *et al.*, *ibid.*, **72**, 1710 (1950).

(7) Micro series reaction vessel. American Instrument Co., Silver Springs, Maryland.

(8) H. Adkins, "Organic Syntheses," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 144.

(9) B. M. Tolbert, *et al.*, *J. Org. Chem.*, **14**, 527 (1949).

of silver sulfate and 30 ml. of water added. A distillation head and condenser were attached and the labeled nitrile was slowly distilled out.

To the distillate 15 g. of potassium hydroxide was added and the solution was allowed to reflux for 24 hours. The mixture was acidified with 50 ml. of 10 *N* sulfuric acid and the acid steam distilled to give 0.949 g. of sodium isocaproate-2-C^{14} with a specific activity of 2.90 ± 0.1 μc./mg. (calculated 3.01 ± 0.1 μc./mg.); yield 55% based on isoamyl bromide used.

Leucine-2-C^{14} (a) Bromination of Isocaproic-2-C^{14} Acid.—Sodium isocaproate-2-C^{14} (0.508 g., specific activity 2.90 μc./mg.) was placed in a Pyrex tube which was connected to a spiral trap (see Fig. 1) and the salt and equipment were dried *in vacuo* at room temperature. The system was disconnected from the vacuum line and the trap immersed in a cooling bath at −20°. Dry hydrogen chloride gas was then passed slowly over the sodium isocaproate which was heated gently. The isocaproic acid thus formed was condensed in the spiral trap. At the completion of this reaction the trap was connected to a bromination vessel (Fig. 1). The trap and the vessel were cooled with liquid air and evacuated to a pressure of 10 microns. Then the free acid was distilled into the cooled bromination vessel by heating the trap to 90–95°.

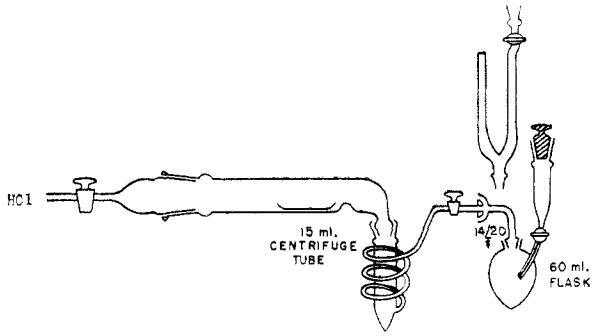

Fig. 1.—Acid generation unit and bromination vessel with condenser separated.

Inactive isocaproic acid (2.7 millimoles) and 0.2 ml. of phosphorus tribromide were added and the mixture allowed to stand overnight. After addition of 0.04 g. of red phosphorus and 0.02 g. of iodine, the condenser was fitted with a protecting Drierite tube and the cold finger was filled with Dry Ice. The bromination flask was heated on a steam-bath for 30 minutes during which 2.3 ml. of bromine was added dropwise.

The bromination flask was then cooled in liquid air and the condenser warmed with hot water. After everything had distilled into the bromination flask, 5 ml. of water was added to the vessel and the mixture was slowly warmed to room temperature. Five more ml. of water was added and the vessel was air-swept for four hours to remove bromine. The product was then extracted with methylene chloride and the resulting solution pressure filtered; this process was repeated several times to give a total volume of solution of about 25 ml.

(b) Amination of the α-Bromoisocaproic-2-C^{14} Acid.—The clean methylene chloride solution was transferred to an ignition tube and evaporated to dryness on the steam-bath; the last traces of solvent were removed by use of the water-pump at room temperature. Then 10 ml. of liquid ammonia was condensed in the tube, the tube sealed and left at room temperature for 40 hours. The tube was then chilled, opened and the excess ammonia evaporated off.

The residue was dissolved in 6 *N* hydrochloric acid and evaporated to dryness. The residue was dissolved in 95% ethanol, filtered and ethylene oxide was passed through the solution for 20 minutes. The precipitate was recrystallized three times from water, using a decolorizing charcoal the first two times.

The yield was 308.6 mg. *Anal.* Calcd. for $C_6H_{13}NO_2$: N, 10.69; specific activity, 1.76 ± 0.1 μc./mg. Found: N, 10.55, 10.53; specific activity, 1.85 ± 0.1 μc./mg. From the mother liquors an additional 121.4 mg. was obtained; specific activity 1.8 μc./mg. Total yield 52% based on isocaproic acid or 20.3% based on barium carbonate used.

Leucine-1-C^{14}.—By the procedure just described, sodium isocaproate-1-C^{14} (1.93 g., 1.70 ± 0.1 μc./mg.) was converted to DL-leucine-1-C^{14} (790 mg., specific activity 1.71 ± 0.1 μc./mg.). The yield was 42% based on isocaproate used or 40% based on starting barium carbonate.

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BERKELEY, CALIFORNIA